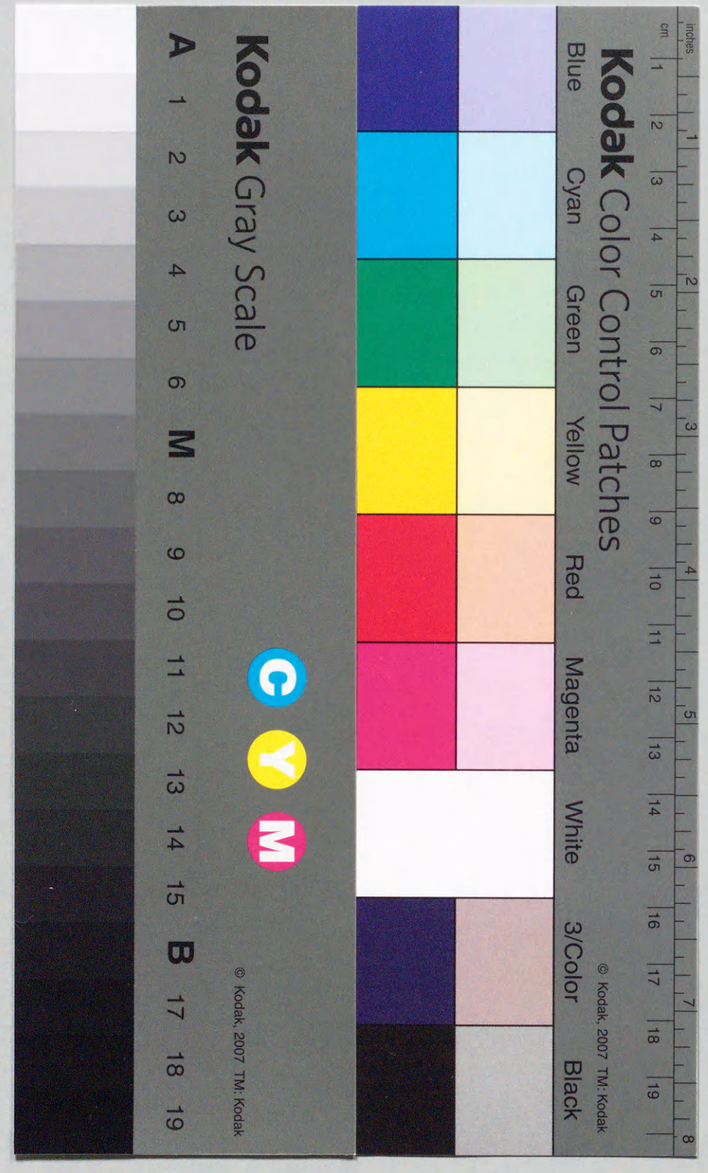

Cytokeratin Polypeptide Profile of Merkel Cells in Human Fetal and Adult Skin: Difference of Expression of Cytokeratins in Epidermal and Dermal Merkel Cells

Yutaka Narisawa, Ken Hashimoto, TaJuana Bayless, Yoshimichi Nihei, Masahiko Ishihara, Dwayne Lawrence, Hikaru Eto, and Keiichiro Hori

Department of Dermatology and Syphilology (YN, KH, TB, YN, MI, HE, KH) and Department of Pathology (DL), Wayne State University School of Medicine, Detroit; and VA Medical Center, Allen Park, Michigan, U.S.A.

The origin of Merkel cells is uncertain, although current evidence by immunohistochemical keratin marker studies favors an epidermal derivation. We studied the expression of keratin species in Merkel cells of human fetus and adult using 19 anti-keratin antibodies. Epidermal and dermal Merkel cells contained not only simple epithelium-type but also some stratified epithelium-type keratins. Interestingly, expression of some keratins was different between epidermal and dermal Merkel cells, for example, AN3 (50, 58 kD) and CKB1 (50 kD) recognized epidermal Merkel cells, but not dermal Merkel cells. These results suggest that surrounding keratinocytes influence the expression of cytokeratins in Merkel cells or that dermal Merkel cells undergo a modification from keratin-producing epidermal Merkel cells to a more neural cell type by the association with nerve endings in the upper dermis. On the other hand, certain cytokeratin polypeptides recognizable with Ks19.1 (40 kD), CK5 (45 kD), and CAM5.2 (52.5 kD) were expressed in both epidermal and dermal Merkel cells. The expression of simple epithelium-type keratins in Merkel cells remained even after the epidermal basal cells gradually lost their expression. *J Invest Dermatol 98:171–180, 1992*

Merkel cells express epithelial features, such as the presence of desmosomes and cytokeratin filaments [1–6]. On the other hand, the cytoplasmic accumulation of membrane-bound, dense-cored vesicles resembling the neurosecretory granules suggests the presence of features of typical neuroendocrine cells [7–11]. In the fetal dermis, Merkel cells are associated with unmyelinated small nerves and in some instances nerve-associated Merkel cells were observed crossing the basal lamina [12]. Such pictures could either be interpreted as the migration of dermal Merkel cells into the epidermis via peripheral nerve or epidermal Merkel cells' emigration into the dermis to couple with peripheral nerves.

We have recently shown that in plantar skin of human fetuses Merkel cells develop in the epidermis when peripheral nerves have not reached the epidermis and there are no detectable Merkel cells in the entire dermis [13]. It was concluded that Merkel cells do not arrive at the epidermis with peripheral nerves and that they may very well originate in the fetal epidermis [13].

The study by Moll et al [2] showed that Merkel cells express simple epithelium-type cytokeratins. The family of cytokeratins consists of at least 19 different proteins [14]. In the present study we investigated the expression patterns of keratin proteins of Merkel cell in fetal and adult skin in order to catalog the expression pattern of these keratin species.

MATERIALS AND METHODS

Tissue Fifteen human fetuses were obtained during iatrogenic abortions performed for medical and nonmedical reasons. Consent forms and collecting practices were approved by the Human and Animal Investigation Committee of Wayne State University.

The estimated gestational ages (EGA) were determined from heel-toe length standards [15,16] and ranged between 11 and 23 weeks of gestation (11 weeks, 2 cases; 12 weeks, 2 cases; 13 weeks, 2 cases; 14 weeks, 1 case; 15 weeks, 2 cases; 16 weeks, 1 case; 18 weeks, 1 case; 19 weeks, 2 cases; 20 weeks, 1 case; and 23 weeks, 1 case). Two adult tissue specimens were obtained from autopsy materials. Tissue samples from the soles of the feet of each specimen were excised immediately and embedded in O.C.T. compound (Lab-Tek Products, Naperville, IL), snap-frozen in liquid nitrogen, and stored at −70°C until use.

Antibodies Nineteen murine monoclonal antibodies specific for epithelial cytokeratins and epithelial membrane antigen were used (Table I).

Immunohistochemistry Frozen sections of human fetal and adult plantar skin were immunostained for epithelial cytokeratins using the avidin-biotin-peroxidase complex (ABC) (Vector Kit, Vector Laboratories, Burlingame, CA) and diaminobenzidine coloration technique [31]. Frozen tissue specimens were cut into 6-μm sections in a cryostat and fixed in cold acetone for 10 min. After incubation with the primary antibody, sections were incubated with biotinylated secondary antibody and avidin-biotin-peroxidase com-

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Reprint requests to: Dr. Ken Hashimoto, Department of Dermatology, Wayne State University, School of Medicine, 540 E. Canfield, Detroit, MI 48201.

Abbreviations:
DAB: diaminobenzidine
EGA: estimate age of gestation
EMA: epithelial membrane antigen

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Table I. Monoclonal Mouse Antibodies Used

Antibody	Specificities	Reference and Source
AE1	50, 56 kD	[17] Signet
AE3	50, 58, 65, 67 kD	[17] Signet
Ks13.1	48, 50, 54 kD	[18] ICN
Ks19.1	40 kD	[19] ICN
MA902	54 kD	[20] Enzo
MA903	56, 56.5, 58, 68 kD	[20] Enzo
MA904	68 kD	[20] Enzo
K8.13	45, 52.5, 54, 56, 56.5, 58, 68 kD	[21] ICN
K8.60	56, 56.5 kD	[22] ICN
CK5	45 kD	[23] Sigma
CAM5.2	52.5 kD	[24] Becton Dickinson
K92	55, 56 kD	[25] Dakopatts
CKB1	50 kD	[26] Sigma
EMA	E29, glycoproteins (265–400 kD)	[27] Dakopatts
AN3	50, 58 kD	[28] Wayne State University
EKH5	Not defined Eccrine gland (secretory)	[29] Wayne State University
EKH6	Not defined Eccrine gland (secretory and coiled duct)	[29] Wayne State University
HKN2	56.5, 63, 72 kD	[30] Niigata University
HKN4	56.5, 58, 66, 67, 72, 76 kD	[30] Niigata University
HKN6	63, 72, 76 kD	[30] Niigata University

plex. The antigen-antibody binding was visualized with 3,3′-diaminobenzidine-tetrahydrochloride (DAB) (Sigma, St. Louis, MO) to produce a dark-brown reaction product. After washes with distilled water, the sections were counterstained with hematoxylin.

Double-Immunoenzyme Labeling When Merkel cells were not distinguished from the surrounding keratinocytes at the single-cell level using the ABC tchnique, the double-immunoenzyme staining method with alkaline phosphatase and peroxidase as labels was carried out. Epithelial membrane antigen (EMA) (DAKO) was useful as a marker of Merkel cells because the staining pattern of EMA was membranous, whereas that of epithelial cytokeratins was cytoplasmic. EMA was confirmed to stain epidermal and dermal Merkel cells of all stages by preliminary experiments.

For double-immunoenzyme staining, the sections were first processed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique [32]. They were incubated with primary antibodies against epithelial cytokeratins for 30 min at room temperature. After washing in tris-buffered saline (TBS), they were reincubated with rabbit immunoglobulin to mouse immunoglobulin (DAKO) for 30 min. After washing in TBS, they were incubated with soluble complex of alkaline phosphatase and monoclonal mouse anti-alkaline phosphatase (DAKO) for 30 min. Subsequently, the enzyme label was developed with Fast Red (final color rendition: red). Afterward, they were immunostained for EMA using the ABC technique with peroxidase conjugate. The peroxidase activity was visualized by 4-chloro-1-naphthol (final reaction product: blue-black) or DAB (final reaction product: dark brown).

Immunoelectron Microscopy In order to confirm that those cytokeratin-positive cells were really Merkel cells, CAM 5.2-stained cells were observed with an electron microscope. The fingers of a freshly aborted 18-week-old fetus were cut and immersed in the mixture of 0.1% glutaraldehyde, 2% paraformaldehyde, and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4 for 3 h. After washing with 0.1 M phosphate buffer with 3.5% sucrose, the residual aldehyde was quenched with 50 mM ammonium chloride in 0.1 M phosphate buffer with 3.5% sucrose for 1 h. Thereafter, the specimens were used in a series of alcohol dehydration processes, namely, 50% alcohol at 4°C, and 95% alcohol at −20°C, twice, 10–20 min each. Then, they were immersed in a mixture of Lowicryl K4 M (Chemische Werke Lowi, Waldkraiburg, FRG) and 95% alcohol (6:4) for 3 h at −20°C. After putting them in 100% Lowicryl K4 M, twice, they were mounted in gelatin capsules and cured with a 366-nm UV lamp for 48 h at −20°C. After curing, ultrathin sections were cut and stained with an indirect method: the meshes were immersed in 5% normal goat serum containing 0.1 M Tris-maleate buffer saline (TMS), pH 7.0, for 30 min. The primary antibody, CAM5.2 (Becton Dickinson, Mountain View, CA), was applied on the mesh for 12 h at 1:20 dilution. After washing with TMS three times, gold-conjugated anti-mouse IgG goat antibody (Sigma Chemical Co., St. Louis, MO) was reacted on the meshes for 3 h. The average particle size of gold was 10 nm and the dilution was 1:50. The meshes were washed and stained with uranyl acetate and lead citrate, and observed with a Hitachi H-300 electron microscope.

RESULTS

The expression patterns of keratin proteins in human Merkel cells and plantar epidermis at different stages of development are summarized in Tables II, III, and IV.

In 11-week-old fetuses the plantar epidermis consisted of two or three cell layers. Any antibodies used did not distinguish Merkel cells at the single-cell level (Fig 1). Even the best markers of Merkel cells in more mature specimens such as CK5, CAM5.2, and EMA were unable to identify the epidermal Merkel cells because these antibodies also stained the basal cells.

In 12-week-old fetuses monoclonal antibodies CK5 and CAM5.2, which label simple epithelium-type 45- and 52.5-kD cytokeratins respectively (Table I) stained individual Merkel cells in and just above the basal layers of plantar epidermis. EKH5, which labels sweat gland mature keratin, and EMA showed the same staining pattern. These were the earliest markers of epidermal Merkel cells (Fig 2). Monoclonal CK5 and CAM5.2-reactive cells were not found in the dermis.

In 13-week-old fetuses, AE3, which recognizes adult epidermal keratin, also labeled the Merkel cells in addition to CK5, CAM5.2, EKH5, and EMA. In 14-week-old fetuses MA902 and EKH6 also recognized the Merkel cells. These are the markers of mature adult keratins. There was no antibody that selectively decorated dermal Merkel cells at this age.

Between 15 and 23 weeks, monoclonal antibodies CK5, CAM5.2, EKH5, EMA, AE1, Ks19.1, MA902, and EKH6 selectively stained both epidermal and dermal Merkel cells in the plantar skin (Figs 2 and 3). The sequential appearances of different species of keratin were also observed in the dermal Merkel cells (Table II). In 16-week-old fetuses AE3, MA903, K8.13, HKN2, HKN4, CK5, CAM5.2, EKH5, EMA, AE1, Ks19.1, MA902, and EKH6 could detect dermal Merkel cells (Table II). The reactivity of these antikeratin antibodies in dermal Merkel cells persisted until 23 weeks, the oldest specimen examined in this study (Table II). The population of dermal Merkel cells peaked at 19 weeks, as reported previously [13]. CKB1 and AN3 recognized only epidermal Merkel cells with a rare exception (Table II). Ks13.1, MA904, K8.60, K92, and HKN6 decorated neither epidermal not dermal Merkel cells (Table II). These results showed that there were differences in the time of expression of the cytokeratins detected by these monoclonal antibodies (Table III).

Monoclonal antibodies AE3, MA903, K8.13, HKN2, and HKN4 consistently stained the entire epidermis throughout fetal development and in adult epidermis (Table IV) (Fig 3). In 11-week-old fetuses CKB1 weakly stained some basal cells. In 12-week-old and older specimens CKB1 stained basal and lower suprabasal layers. In 15-week-old fetuses AN3 stained the entire epidermis. In 16-week-old and older specimens, AN3 stained basal and lower suprabasal layers (Fig 3). Thus, in contrast to the monoclonal antibodies listed in Table IV, these antibodies were unable to differentiate Merkel cells from basal and suprabasal keratinocytes in the plantar epidermis. It was important to know whether or not these antibodies actually recognized the Merkel cells. The double-labeling method using EMA as a marker of epidermal and dermal Merkel cells confirmed the presence of merkel cells because EMA did not stain keratinocytes in the overlying epidermis or dermal connective tissue cells after a 12-week EGA. Cells labeled red in the cytoplasm with monoclonal antibodies AE3, MA903, K8.13, HKN2, and HKN4 with APAAP and Fast Red were simultaneously labeled brown with EMA in their cell membrane using the peroxidase-diaminobenzidine color development technique. When monoclonal antibodies CKB1 and AN3 were applied, epidermal Merkel cells showed double staining of cytokeratins with these and cell membrane with EMA. However, most of the dermal Merkel cells lacked reactivity to the cytokeratins. In 16-week-old fetuses, the double-labeling method with AN3 and EMA demonstrated EMA-positive dermal Merkel cells with and without AN3 reactivity (Fig 4). Fetuses between 18 and 23 weeks had EMA-positive dermal Merkel cells that

Table II. Reactivity of Keratin Filament Antibodies and Epithelial Membrane Antigen in Human Fetal and Adult Merkel Cells

		Fetus							
		11 Weeks	12 Weeks	13 Weeks	14 Weeks	16 Weeks	20 Weeks	23 Weeks	Adult
CK5	E[a]	–	+[c]	+	+	+	+	+	+
	D[b]	–	–	–	–	+	+	+	–
CAM5.2	E	–	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	–	–
EKH5	E	–	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
EMA	E	–	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
AE1	E	–	–	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
Ks19.1	E	–	–	–	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
MA902	E	–	–	–	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
EKH6	E	–	–	–	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
Ks13.1	E	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–
MA904	E	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–
K8.60	E	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–
K92	E	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–
HKH6	E	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–
AE3	E	?[d]	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
MA903	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
K8.13	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
HKN2	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
HKN4	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	+	+	+	–
CKB1	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	–	–	–	–
AN3	E	?	+	+	+	+	+	+	+
	D	–	–	–	–	(+)[e]	–	–	–

[a] E, epidermis.
[b] D, dermis.
[c] +, Merkel cells could be distinguished from keratinocytes on normal connective tissue cells.
[d] ?, all antibodies stained basal cells and Merkel cells could not be identified selectively. Therefore, it is difficult to determine whether Merkel cells are present or absent at this stage.
[e] (+), only one dermal Merkel cell was labeled with AN3.

Table III. Sequential Expression of Keratin Epitopes of Epidermal Merkel Cell

11th Week	No Immunoreactive Merkel cells
12th Week	Simple epithelium keratins CK5, CAM5.2, EKH5, (EMA)
	Simple and stratified epithelium keratins AE3, MA903
	Stratified epithelium keratins AN3, HKN2, HKN4, etc
13th Week	Simple epithelium keratins as above
	Simple and stratified epithelium as above
	Stratified epithelium keratins as above
14th Week	Simple epithelium keratins as above and MA90 (54 Kd)
	Simple and stratified keratins as above and EKH6
	Stratified keratins as above
15th–23rd Week	All of the above

Table IV. Antikeratin Antibodies That Label the Entire Epidermis Throughout the Fetal Development and in Adult Life; not Useful for Merkel Cell Detection in the Epidermis

		Fetus					
		11 Weeks	12 Weeks	14 Weeks	20 Weeks	23 Weeks	Adult
AE3	P[a]	+[d]	+	+			
	SB[b]	/	+	+	+	+	+
	B[c]	+	+	+	+	+	+
MA903	P	+	+	+			
	SB	/	+	+	+	+	+
	B	+	+	+	+	+	+
K8.13	P	+	+	+			
	SB	/	+	+	+	+	+
	B	+	+	+	+	+	+
HKN2	P	+	+	+			
	SB	/	+	+	+	+	+
	B	+	+	+	+	+	+
HKN4	P	+	+	+			
	SB	/[h]	+	+	+	+	+
	B	+	+	+	+	+	+
CKB1	P	−[g]	−	−			
	SB	−	±[f]	(+)[e]	(+)	(+)	(+)
	B	±	+	+	+	+	+
AN3	P	+	+	+			
	SB	/	+	+	(+)	(+)	(+)
	B	+	+	+	+	+	+

[a] P, periderm.
[b] SB, suprabasal.
[c] B, basal.
[d] +, positive reaction.
[e] (+), staining of lower suprabasal layer.
[f] ±, weak reaction.
[g] −, no reaction.
[h] /, in 11-week-old fetuses it was difficult to distinguish intermediate cells between basal cells and periderm.

totally lacked the reactivity with CKB1 and AN3. CKB1 and AN3 were positive in the epidermal Merkel cells from 12 to 23 EGA (Table II). All other keratin markers that were reactive in the epidermal Merkel cells eventually became positive in the dermal Merkel cells (Table II). Electron microscopy has abundantly demonstrated that the epidermal Merkel cells cross the basal lamina [12,13] and this phenomenon is now interpreted as evidence of the emigration of epidermal Merkel cells into dermis. Thus, the total absence of CKB1 and a partial absence of AN3 in dermal Merkel cells seem to suggest a cessation of the expression of cytokeratin proteins reactive with CKB1 and AN3 by 18 EGA or earlier.

In the 11-week-old fetuses Ks19.1 stained the periderm and basal cells. In the 14-week-old fetuses the staining of the basal layer with Ks19.1 diminished and as a result only Merkel cells were decorated in some areas (Fig 5). In the 16-week-old fetuses epidermal basal cells were not stained with Ks19.1 and only Merkel cells remained positive. Expression of CK5, CAM5.2, AE1, MA902, and EKH6 followed the pattern of Ks19.1 (Table V). These data suggest that Merkel cells contain essentially the same intermediate-sized keratin filments as basal cells at an early stage of development and retain them long after the basal cells switched to produce more mature type keratins. In our panel of anti-keratin antibodies, there was no antibody that recognized only Merkel cells throughout fetal and adult skin development.

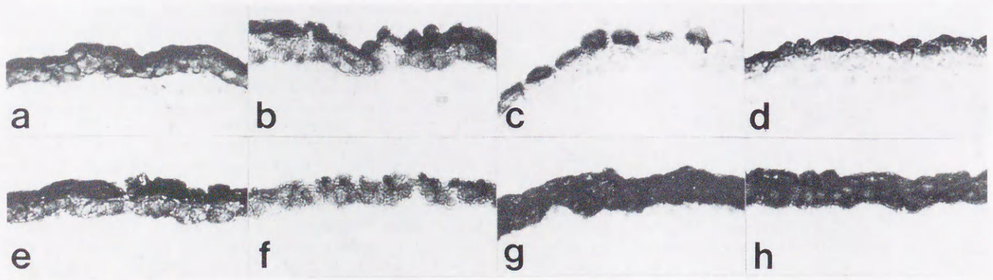


Figure 1. Plantar skin of 11-week-old human fetus after incubation with *(a)* CK5; *(b)* CAM5.2; *(c)* EKH5; *(d)* EMA; *(e)* AE1; *(f)* AE3; *(g)* MA903, and *(h)* AN3. EKH5 and EMA weakly stain basal cells. All antibodies heavily label periderm and basal cells. Any antibody cannot differentiate Merkel cells at the single-cell level. No counterstain. Peroxidase/DAB. Magnification ×160.

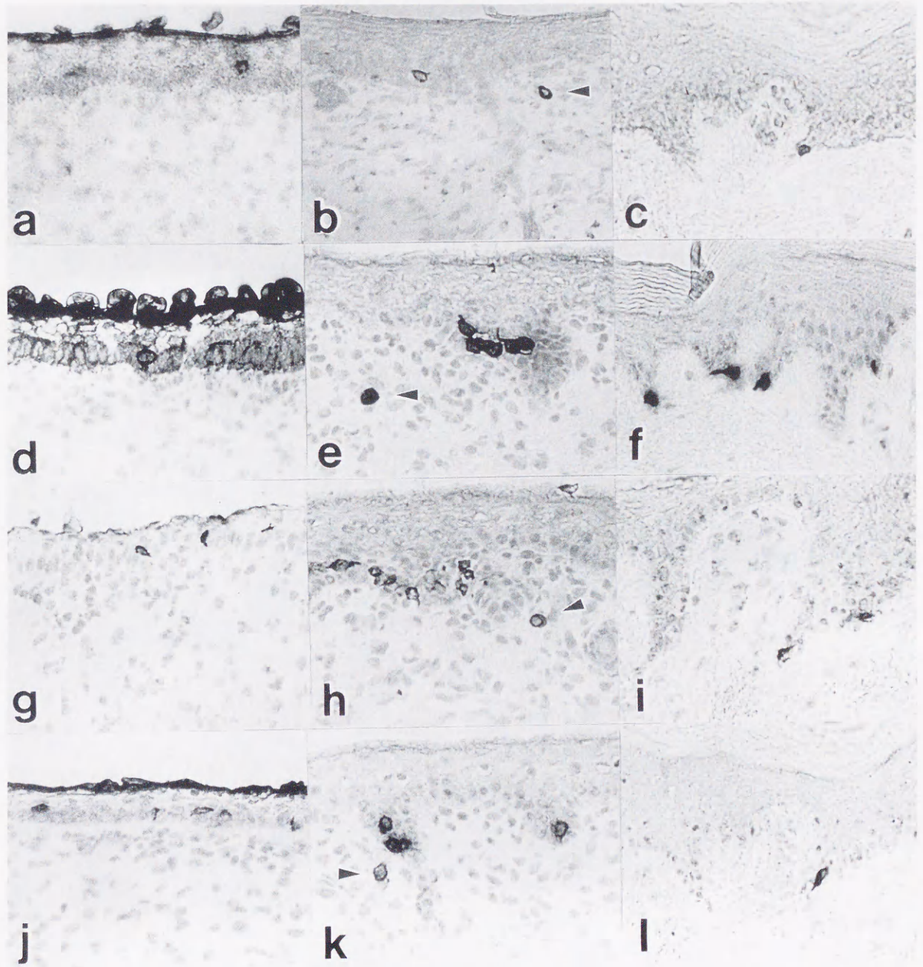


Figure 2. Plantar skin of 12 *(a,d,g,j)*- and 20 *(b,e,h,k)*-week-old human fetus and adult *(c,f,i,l)*. CK5 *(a,b,c)*, CAM5.2 *(d,e,f)*, EKH5 *(g,h,i)*, and EMA *(j,k,l)* selectively decorate epidermal and dermal Merkel cells *(arrowhead)*. Hematoxylin counterstain. Staining method is same as for Fig 1. Magnification ×160.

Immunoelectron microscopy using CAM5.2 as a simple epithelial keratin marker identified that those CAM5.2-positive cells are indeed Merkel cells because of the presence of the specific Merkel cell granules in their cytoplasm (Figs 6 and 7).

DISCUSSION

It has recently been shown by immunohistochemistry that mammalian Merkel cells contain neuron-specific enolase [33], synaptophysin [34], chromogranin A [35], vasoactive intestinal polypeptides (VIP) [36], met-enkephalin [37], serotonin [38], pancreastatin [39], and calcitonin gene-related peptide [40]. These findings justified the classification of mammalian Merkel cells with the diffuse neuroendocrine system (DNES) [41].

On the other hand, Merkel cells also express cytokeratin polypeptides demonstrating their epithelial cell character [2]. Previous studies have shown that Merkel cells contain simple epithelium-type keratins and lack the keratin polypeptides specific for adult keratinocytes. In this report we demonstrated that Merkel cells contain not only simple embryonic epithelium-type, but also stratified adult epithelium-type keratins, although some of these mature keratins, such as those recognizable with KA1, were reported to be absent [42]. Also, we found that there were time differences for the expression of each keratin species. For example, CAM5.2 (52.5 Kd)- and CK5 (45 Kd)-positive Merkel cells were already found in 12-week-old fetuses. The reactivity of Ks19.1 was observed first in 14-week-old fetuses in all basal cells; it gradually decreased and eventually disappeared while the reactivity remained only in Merkel cells. This

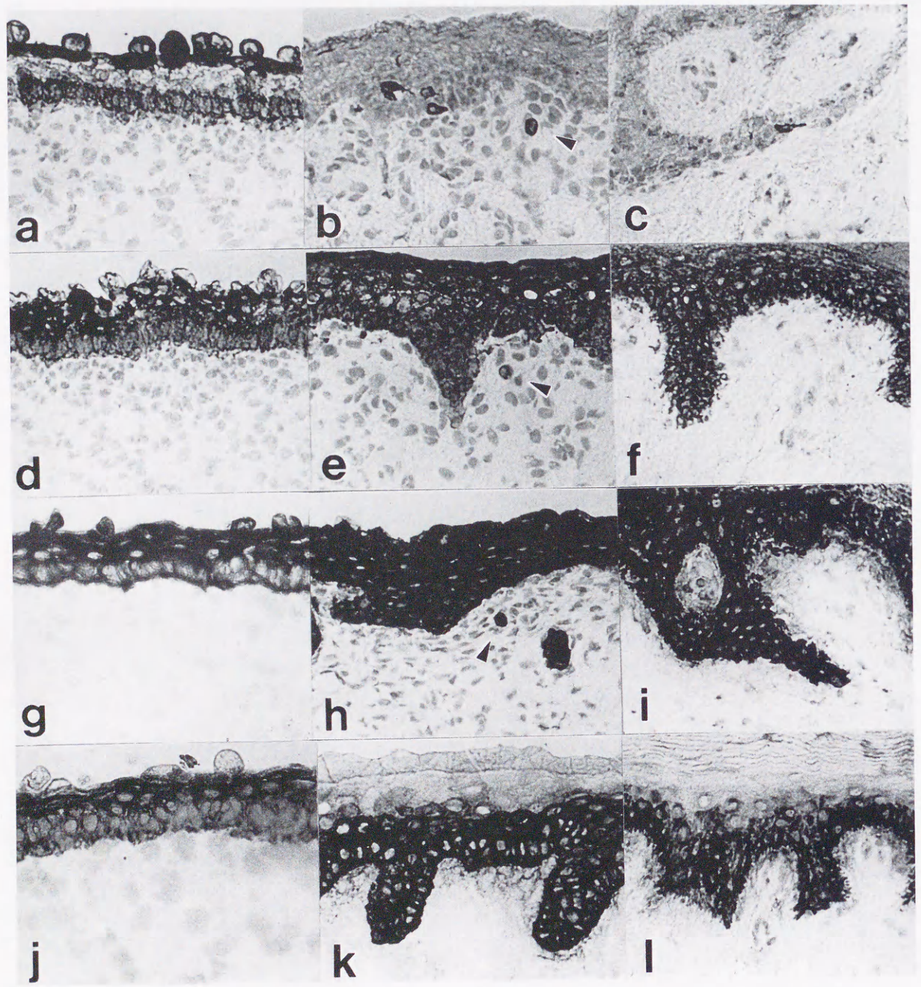


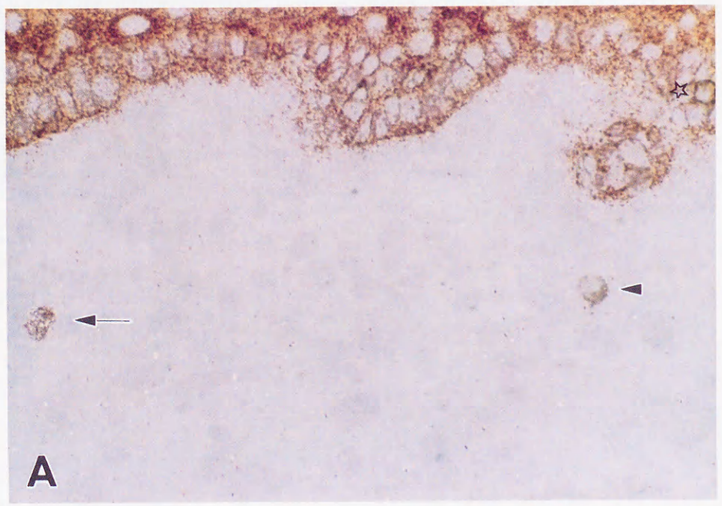
Figure 3. Plantar skin of 12 *(a,d,g,j)*- and 20 *(b,e,h,k)*-week-old human fetus and adult *(c,f,i,l)*. AE1 *(a,b,c)*, AE3 *(d,e,f)*, and MA903 *(g,h,i)* decorate dermal Merkel cells *(arrowhead)*, but not AN3 *(j,k,l)* in 20-week-old human fetus. Hematoxylin counterstain. Staining method is same as for Fig 1. Magnification ×160.

fading pattern of Ks19.1 was also observed with CK5, CAM5.2, EKH5, AE1, MA902, and EKH6.

Some antikeratin antibodies such as CKB1 and AN3 decorated only epidermal Merkel cells. In such instances dermal Merkel cells never expressed these keratins (except one AN3-positive cell shown in Fig 4) despite the fact that EMA-positive dermal Merkel cells were definitely present in the dermis of the same specimen. In the early (16-week) stage we found only one dermal Merkel cell with AN-3 reactivity, but not in later stages (18–23 weeks). No CKB1-positive dermal merkel cell was found at any age. The findings suggest that dermal merkel cells ceased to express or never developed CKB1 (50 Kd)- and AN3 (50, 58 Kd)-reactive, 50- and/or 58-kD keratins. A pair of 50- and 58-kD keratins are typically found in stratified epithelium [43]. It is speculated that the expression of certain cytokeratins is necessary for epidermal merkel cells to interact with surrounding keratinocytes or that the differentiation of dermal Merkel cells is switched more toward the neural direction.

In our examination, EKH-5 completely lacked reactivity with basal cells throughout human fetal and adult life. Except for its reactivity with periderm cells, it is the most specific, exclusive Merkel cell marker in the epidermis.

Finally, in all previous works [2,6,14,18] simple epithelium keratin-positive epidermal and dermal cells have been presumed to repre-



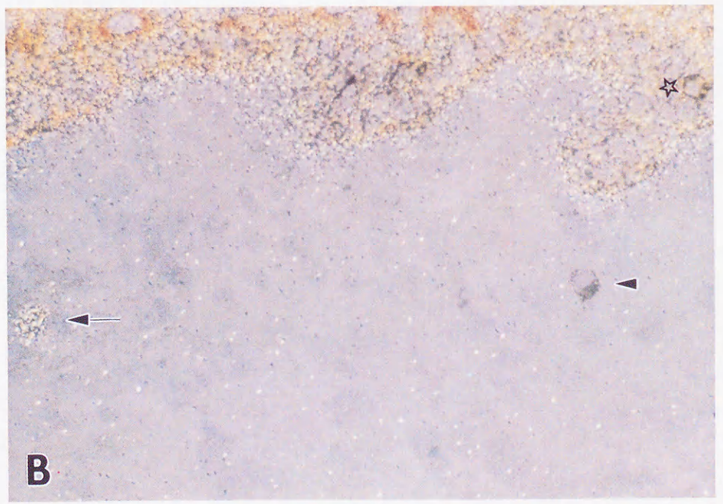


Figure 4. Double-immunoenzyme labeling for EMA and AN3 of plantar skin of 16-week-old human fetus. *A*, AN3/APAAP/Fast red/red. EMA/peroxidase/DAB/dark brown. Here, red-brown deposit reveals the AN3- and EMA-positive dermal Merkel cell *(arrow)*, and blue-brown circle without red deposits shows the EMA-positive, AN3-negative dermal Merkel cell *(arrowhead)*. *B*, phase contrast picture of *A*. Small, dense, refractile particles represent AN3 reactivity. There are two dermal Merkel cells, each with *(arrow)* and without *(arrowhead)* the aggregation of refractile particles, corresponding to AN3-positive and AN3-negative dermal Merkel cells. No counterstain. *, epidermal Merkel cell. Magnification ×250.

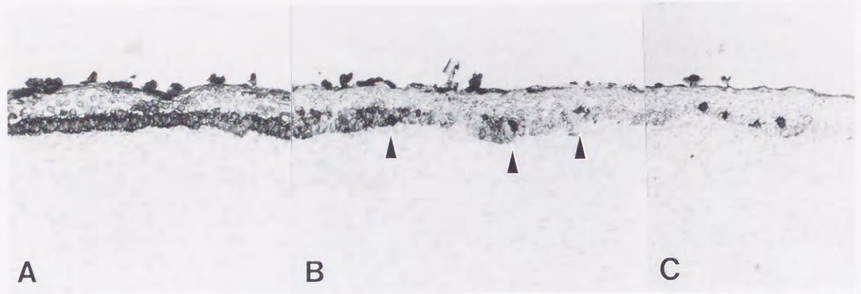


Figure 5. Immunoperoxidase staining patterns of the expression of cytokeratin proteins with Ks19.1 (40 Kd) in plantar skin of a 14-week-old human fetus. *A*, In this area Ks19.1 strongly labels periderm and basal cells. Merkel cells are not differentiated from the keratinocytes or masked by a strong staining of keratinocytes. *B*, *C*, The stainability of basal cells decreased and Merkel cells are gradually distinguished just above the basal layer. *A*, *B*, *C*, same section. No counterstain. Staining methods is same as for Fig 1. Magnification ×128.

Table V. Antikeratin Antibodies That Label Entire Epidermis in Early Fetal Life and Cease to be Reactive in Basal Keratinocytes; Useful for Merkel Cell Detection in the Epidermis

		Fetus					
		11 Weeks	12 Weeks	14 Weeks	20 Weeks	23 Weeks	Adult
CK5	P[a]	+[d]	+	+			
	SB[b]	/[g]	−[f]	−	−	−	−
	B[c]	+	±	−	−	−	−
CAM5.2	P	+	+	+			
	SB	/	−	−	−	−	−
	B	+	±	−	−	−	−
EKH5	P	+	+	+			
	SB	/	−	−	−	−	−
	B	+ ~ ±	−	−	−	−	−
EMA	P	+	+	+			
	SB	/	−	−	−	−	−
	B	+ ~ −	± ~ −	−	−	−	−
AE1	P	+	+	+			
	SB	/	±	−	−	−	−
	B	+	+ ~ ±	+ ~ ±	−	−	− ~ +
Ks19.1	P	+	+	+			
	SB	/	+	± ~ −	−	−	−
	B	+	+	+ ~ −	−	−	−
MA902	P	+	+	+			
	SB	/	+	±	−	−	−
	B	+	+	±	−	−	−
EKH6	P	+	+	+			
	SB	/	+	+	+	+	+
	B	+	+	±	−	−	−
Ks13.1	P	+	+	+			
	SB	/	+	+	+	+	+
	B	−	−	−	−	−	− ~ +
MA904	P	−	−	−			
	SB	±	+	+	+	+	+
	B	−	−	−	−	−	−
K8.60	P	−	−	−			
	SB	±[e]	+	+	+	+	+
	B	−	−	−	−	−	−
K92	P	−	−	−			
	SB	±	+	+	+	+	+
	B	−	−	−	−	−	−
HKN	P	+	+	+			
	SB	/	+	+	+	+	+
	B	+	±	±	±	±	±

[a] P, periderm.
[b] SB, suprabasal.
[c] B, basal.
[d] +, positive reaction.
[e] ±, weak reaction.
[f] −, no reaction.
[g] /, in 11-week-old fetuses it was difficult to distinguish intermediate cells between basal cells and periderm.

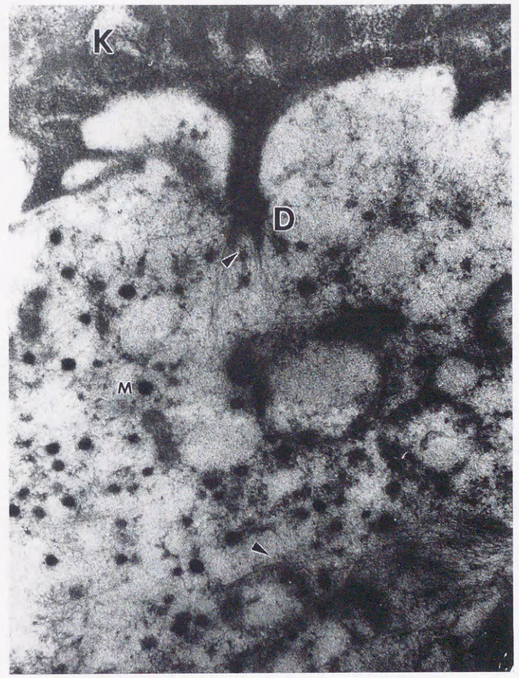


Figure 6. Immunoelectron microscopic demonstration of CAM5.2 staining of intermediate filaments in an epidermal Merkel cell of 18-week-old fetus. Gold particles *(arrowheads)* indicate the sites of CAM5.2 labeling. This cell is identified as Merkel cell by the presence of specific Merkel cell granules (M) and a desmosomal connection (D) to the adjacent keratinocyte (K). Magnification ×24,000.

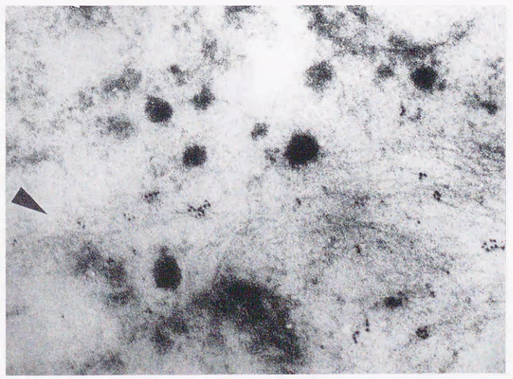


Figure 7. High magnifications of immunogold depositions on keratin filaments in the areas designated by the *lower arrowhead* in Fig 6. Magnification ×96,000.

sent the Merkel cells. In this study we have demonstrated that CAM5.2-positive epidermal cells are indeed Merkel cells by identifying Merkel cell granules and CAM5.2-positive keratin filaments in such cells using the immunogold technique at the ultra-structural level.

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